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Description

[0001] This invention relates to angiogenesis inhibitory antibodies, and to the use thereof in the inhibition of angiogenesis, particularly angiogenesis associated with the growth of solid tumours, with proliferative retinopathies, and with certain inflammatory diseases.

[0002] The circulatory system represents an extensive, branching, network of blood vessels which is essential for the supply of oxygen and nutrients to tissues and for the removal of byproducts of metabolism. In adults the development of new blood vessels or "angiogenesis" rarely occurs except during wound healing or as a result of a number of pathological situations termed "angiogenesis-dependent diseases"[1,2]. The most important of these is the angiogenesis associated with the growth of solid tumours and with proliferative retinopathies. Angiogenesis may also play an important role in rheumatoid arthritis and psoriasis.

[0003] Angiogenesis inhibitors can, therefore, be of considerable value in the treatment of angiogenesis-dependent diseases. For example, in the case of solid tumours, the development of a blood supply is essential for the growth and survival of the tumour. Thus, inhibition of angiogenesis can provide a highly selective means of inducing tumour regression. Similarly, angiogenesis inhibitors may be used to prevent the blindness associated with proliferative diabetic retinopathy, one of the major complications of diabetes.

[0004] In work leading to the present invention, monoclonal antibodies (mAbs) have been developed against proliferating/angiogenic human endothelial cells which can be used either to directly inhibit angiogenesis or to target cytotoxic drugs or radioisotope labels to sites of angiogenesis. Since angiogenesis does not occur in adults, except following tissue injury, such mAbs can be remarkably specific. Furthermore, unlike other lines of research which have produced cancer cell-specific mAbs to target cytotoxic drugs to tumours, the present invention is directed to producing mAbs against host antigens. This approach has the major advantage that generation of "resistant" variants of the tumour cannot occur and, in theory, one mAb could be used to treat all solid tumours. An additional advantage is that endothelial cells, by virtue of their vascular location, are very accessible to mAbs in the circulation.

[0005] Accordingly the invention provides an antibody which specifically binds proliferating human endothelial cells, said antibody binding proliferating human umbilical vein endothelial cells or proliferating human umbilical artery endothelial cells cultured in medium 199 supplemented with 20% foetal calf serum, L-glutamine, antibiotics, 100 µg/ml heparin and 1.2 µg/ml endothelial cell growth supplement, and not binding non-proliferating human umbilical vein endothelial cells or non-proliferating human umbilical artery endothelial cells.

[0006] The antibody may be a monoclonal antibody.

[0007] This invention also extends to a hybridoma cell line producing the monoclonal antibody of the invention. The cell line may be produced by methods well known to persons skilled in this field.

[0008] As previously described, the antibody of the invention may be used alone as an anti-angiogenesis agent in the treatment of angiogenesis-dependent disease in a patient.

[0009] The invention also provides an antibody-conjugate comprising an antibody of the invention having a toxic material or label conjugated thereto.

[0010] The toxic material may, for example, be cytotoxic. Thus typically the antibody is conjugated to a cytotoxic material. The material may be a cytotoxic drug. Other toxic materials well known to persons skilled in this art may also be incorporated in the antibody-conjugate of the invention. Typically the cytotoxic material is ricin A chain, diphtheria toxin, Pseudomonas exotoxin A or idarubicin.

[0011] Typically the antibody is conjugated to a radioisotope label. A suitable radioisotope label is technetium -99m. Coupling of various toxins to monoclonal antibodies may be effected by known methods[3,4,5,6]. Similarly, the preparation of a conjugate with a radiolabel may use known methods[7].

[0012] The invention also provides a therapeutic composition for treatment of angiogenesis-dependent disease, comprising an antibody or an antibody-conjugate of the invention together with a pharmaceutically acceptable carrier or diluent.

[0013] The invention provides an antibody or antibody conjugate of the invention for use in a method of treating angiogenesis-dependent disease in a patient. Typically for use in a method of inhibiting angiogenesis associated with the growth of solid tumours or with proliferative retinopathies.

[0014] The invention also provides use of an antibody or antibody-conjugate of the invention in the manufacture of a pharmaceutical composition for treatment of angiogenesis-dependent disease. Typically the angiogenesis is angiogenesis associated with the growth of solid tumours or with proliferative retinopathies.

[0015] Administration of the antibody or antibody-conjugate may be by any suitable route. Preferably, the administration to the patient is parenterally, for example, by injection.

[0016] The invention also provides a method for producing an antibody of the invention, comprising raising antibodies against proliferating human umbilical vein endothelial cells (HUVEC), screening the antibodies for proliferating HUVEC reactivity, eliminating antibodies which react with other human cell lines, and identifying an antibody which fails to react with freshly isolated, non-proliferating human endothelial cells.

[0017] In one embodiment of this invention, there have been developed monoclonal antibodies (mAbs) specific for proliferating/angiogenic endothelial cells. The major use of these mAbs is to simply inhibit angiogenesis, although if desired the mAbs can be used to target cytotoxic drugs or labels to angiogenic sites. In the case of tumours, this approach has the major advantages of tumour specificity, minimal side-effects, and little chance of "resistant" tumour variants arising. Furthermore, these mAbs provide a single therapeutic agent that can be used for all solid tumours, regardless of type and tissue location, and inhibition of angiogenesis in the solid tumours can result in tumour regression.

[0018] The initial experimental approach has been to raise murine mAbs against proliferating/angiogenic human umbilical vein endothelial cells (HUVEC). Resultant mAbs have been screened initially for HUVEC reactivity and, subsequently, mAbs have been eliminated which react with other human cell lines, e.g. human melanoma cell lines. Finally, endothelial specific mAbs have been identified which fail to react with freshly isolated, non-proliferating/non-angiogenic human endothelial cells. Using this approach, it has been clearly established that mAbs can be obtained which are specific for proliferating/angiogenic human endothelial cells.

BRIEF DESCRIPTION OF THE DRAWING

[0019]

Figure 1 shows binding of mAbs to proliferating/angiogenic and resting (non-proliferating/non-angiogenic) human umbilical vein endothelial cells (HUVEC) as detected by immunofluorescence flow cytometry. CONT refers to HUVEC not incubated with mAbs, 20G5 is a HUVEC-specific mAb which reacts with both proliferating/angiogenic and resting HUVEC and 9B11 is a HUVEC-specific mAb which only reacts with proliferating/angiogenic HUVEC.

[0020] Further details of the present invention will be apparent from the following detailed description of the production of endothelial specific mAbs in accordance with the invention.

EXAMPLE

A. Materials and Methods

Cells

[0021] Human umbilical vein (HUVEC) and artery (HUAEC) endothelial cells were prepared from human umbilical cords by the method of Jaffe[8] and cultured in Medium 199 supplemented with 20% foetal calf serum (FCS), L-glutamine, antibiotics, 130 ug/ml heparin and 1.2 µg/ml endothelial cell growth supplement (Sigma). HUVEC were used for mAb binding studies between passages 2 and 7. Human tumour cell lines (e.g. MM-170 melanoma, K562 erythroleukaemia) were cultured in RPMI-1640/10% FCS. Mononuclear cells (lymphocytes and monocytes) and neutrophils were simultaneously isolated from human peripheral blood by centrifugation of diluted blood on Polymorphprep™ (Nycomed. Pharma A.S., Oslo, Norway). Red cells and platelets were isolated by differential centrifugation from citrated human blood.

Production of Hybridomas

[0022] BALB/c mice were immunised, i.p., 3-4 times at 2-4 weekly intervals with $15x10^6$ HUVEC in PBS and challenged 3 days prior to spleen cell removal with $15x10^6$ HUVEC. A spleen cell suspension was prepared, fused with the myeloma NS1/1.AG4.1 and hybridomas grown up and cloned as described previously[9]. To improve hybridoma growth and cloning efficiencies 10% endothelial cell conditioned medium (HUVEC or bovine corneal EC) was included in culture media.

mAb Screening Assays.

[0023] Initially hybridoma culture supernatants were tested for reactivity with HUVEC by immunofluorescence flow cytometry. Briefly, HUVEC ($5x10^4$) were incubated (30 min, 4°C) with undiluted hybridoma supernatant, washed and incubated with FITC-sheep $F(ab')_2$ anti-mouse Ig(100µg/ml). Following final washing HUVEC were examined for mAb binding by analysis on a Becton-Dickinson FACScan. Positive hybridoma supernatants were then screened on the human melanoma cell line MM-170 to eliminate non-endothelial specific mAbs. Endothelial specificity was further confirmed by screening mAbs on a panel of human tumour cell lines and human lymphocytes, monocytes, neutrophils, red cells and platelets. Finally, specificity for proliferating HUVEC was established by screening hybridoma superna-

tants on freshly isolated (non-cultured) HUVEC. Hybridomas which were positiv on proliferating HUVEC but negativ on freshly isolated HUVEC were cloned[9] for further study. A number of hybridomas (e.g. 20G5) which were endothelial-specific but not proliferation/angiogenesis-sp cific wer also cloned.

HUVEC Proliferation Assay

[0024] Assays were performed in 96 well, flat bottom, microplates coated with 0.1% gelatin and containing $2.5x10^4$ HUVEC/well in 150µl of culture medium. After 24hr culture cells were pulsed with ^3H-thymidine for a further 24hr and ^3H-thymidine incorporation assessed in washed and harvested cells using a Titertek 530 cell harvester (Flow Labs). In mAb blocking experiments 50 µl/well of hybridoma supernatant was added at the commencement of the cultures with supernatant from a hybridoma which does not react with HUVEC being used as a negative control.

B. Results

Production of mAbs Specific for Proliferating/Angiogenic Endothelial Cells

[0025] Table 1 shows that mAbs can be obtained which are specific for proliferating/angiogenic human endothelial cells.

TABLE 1

Production of Endothelial Specific Monoclonal Antibodies (mAbs).		
Hybridomas	Number	
	Fusion #1	Fusion #2
Total screened	1196	660
Proliferating HUVEC positive	811	276
Proliferating HUVEC specific	541[a]	102[b]
Non-proliferating (resting) HUVEC negative	25[c]	17[c]

a Hybridomas not reactive with the human melanoma cell line MM-170.

b Hybridomas not reactive with human MM170 cell line, U937 monocytic cell line, lymphocytes, neutrophils, monocytes, red cells and platelets.

c Hybridomas not reactive with endothelial cells freshly isolated from the human umbilical cord, i.e. endothelial cells "non-proliferating" or "resting".
HUVEC = Human umbilical vein endothelial cells.

[0026] In the first fusion of 1196 hybridomas screened, 811 reacted with proliferating/angiogenic endothelial cells of which 541 were proliferating/angiogenic endothelial cell specific, i.e. failed to react with other proliferating human cell lines such as the human melanoma line MM-170. Of particular importance was the fact that 25 of the 541 hybridomas specific for proliferating/angiogenic human endothelial cells failed to react with non-proliferating/non-angiogenic (freshly isolated) endothelial cells. Thus, 4.6% of hybridomas produce mAbs which are proliferation/angiogenesis specific, a clear validation of the approach being used. A similar result was obtained in a second fusion where 16.6% of the HUVEC-specific mAbs were angiogenesis specific. A typical example of the results obtained with a proliferation/angiogenesis-specific (9B11) and a proliferation/angiogenesis non-specific (20G5) mAb is depicted in Fig.1 as revealed by immunofluorescence flow cytometry.

Table 2.

Reactivity Pattern of Some Cloned Monoclonal Antibodies Against Human Endothelial Cells						
Human Cells	mAb Clones					
	9D9 (IgM)	12E5 (IgM)	10A5 (IgM)	14G11 (IgG1)	21F10 (IgM)	20G5 (IgM)
Proliferating HUVEC	+	+	+	+	+	+
Resting HUVEC	-	-	-	-	-	+
Proliferating HUAEC	+	+	+	+	+	+
K562 erythroleukaemia	-	-	+	+	+	-
MM170 melanoma	-	±	+	+	+	-
PE.01 ovarian carcinoma	-	-	+	+	+	-

Table 2. (continued)

Reactivity Pattern of Some Cloned Monoclonal Antibodies Against Human Endothelial Cells						
Human Cells	mAb Clones					
	9D9 (IgM)	12E5 (IgM)	10A5 (IgM)	14G11 (IgG1)	21F10 (IgM)	20G5 (IgM)
COLO397 colonic carcinoma	-	-	+	+	+	-
KJD keratinocyte carcinoma	-	-	+	+	+	-
MT2 B lymphoma	-	-	+	+	+	+
Molt 4 T lymphoma	-	-	+	+	+	+
U937 (monocytic)	-	-	+	+	+	-
Lymphocytes	-	-	+	-	-	+
Neutrophils	-	±	±	-	-	+
Monocytes	-	+	+	±	-	+
RBC	-	-	-	-	-	-
Platelets	±	-	±	+	-	+
Fibroblasts	-	-	+	±	-	-
HUVEC = human umbilical vein endothelial cells. HUAEC = human umbilical artery endothelial cells.						

[0027] Table 2 presents detailed specificity analysis of six cloned mAbs, which were HUVEC reactive, as examples. One mAb (20G5) is a control which reacts with both resting and proliferating/angiogenic endothelial cells and is probably specific for the CD31 antigen. The remaining five mAbs react with proliferating/angiogenic but not resting endothelial cells. Three of these mAbs (10A5, 14G11, 21F10) react with many other proliferating cell types. The remaining two clones (9D9 and 12E5) exhibit considerable specificity for proliferating/angiogenic endothelial cells, 9D9 being the mAb with the greatest specificity, only exhibiting a weak reaction with platelets.

[0028] The 9D9 mAb reacts with proliferating/angiogenic venular or arterial endothelial cells but not non-proliferating (resting) endothelial cells (Table 2). Subsequent studies showed that the 9D9 antigen appears on cultured HUVECS within 24 hr of culture and persists on HUVEC cultured for many passages, i.e. ten passages over a period of two months. The 9D9 antigen also appears on HUVEC whether they are cultured in 20% FCS + bovine growth supplement or 20% human serum, indicating that the 9D9 antigen is not derived from culture medium components.

Effect of mAbs on Endothelial Cell Proliferation.

[0029] When some of the proliferation-specific mAbs were added to proliferating HUVEC *in vitro* it was found that some of the mAbs could directly inhibit HUVEC proliferation. The results of a typical experiment are present in Table 3.

TABLE 3

Inhibition of HUVEC Proliferation by mAbs Specific for Proliferating/Angiogenic Endothelial Cells.			
mAb	Specificity	^{3}H-Thymidine Incorporation* (cpm)	Response % Control
9B9	Non-reactive	7779±1420	100
20G5	HUVEC	6806±1290	87.5
1D5	Proliferating HUVEC**	1256±110	16.1
8G4	Proliferating HUVEC**	1857±38	23.9
16C6	Proliferating HUVEC**	1767±175	22.7
19D4	Proliferating HUVEC**	7530±753	96.8

* HUVEC cultured in proliferation assay with dialyzed hybridoma supernatants containing mAbs. Proliferation measured 24-48 hr following culture initiation and represents mean ± standard error of three determinations.

** mAbs only react with proliferating/angiogenic (not resting) HUVEC.

[0030] Of the four proliferation/angiogenesis-specific mAbs tested, three (1D5, 8G4 and 16C6) inhibited HUVEC proliferation by approx. 75-85% as measured by ^{3}H-thymidine incorporation. In contrast, one proliferation/angiogen-

esis-specific mAb (19D4) and 20G5, a mAb which reacts with both prolif rating and non-prolif rating HUVEC, had no significant effect on HUVEC proliferation. The mAb 9B9, which does not react with HUVEC, was us d as the n gative control in this experiment.

[0031] Th s data strongly suggest that som of th proliferation/angiog nesis-specific mAbs may directly inhibit angiogenesis, thus bypassing the need for cytotoxic drug-mAb conjugates. It should be emphasised that the data presented in Table 2 were obtained with hybridoma supernatants and not with purified and concentrated mAb preparations.

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Claims

1. An antibody which specifically binds proliferating human endothelial cells, said antibody
   binding proliferating human umbilical vein endothelial cells or proliferating human umbilical artery endothelial cells cultured in medium 199 supplemented with 20% foetal calf serum, L-glutamine, antibiotics, 130 µg/ml heparin and 1.2 µg/ml endothelial cell growth supplement, and
   not binding non-proliferating human umbilical vein endothelial cells or non-proliferating human umbilical artery endothelial cells.
2. An antibody according to claim 1 which is a monoclonal antibody.
3. A hybridoma cell line producing a monoclonal antibody according to claim 2.
4. An antibody-conjugate comprising an antibody as claimed in claim 1 or 2 having a toxic material or label conjugated thereto.
5. An antibody-conjugate according to claim 4, wherein said antibody is conjugated to a cytotoxic material.
6. An antibody-conjugate according to claim 5, wherein said cytotoxic material is ricin A chain, diphtheria toxin, Pseudomonas exotoxin A or idarubicin.
7. An antibody-conjugate according to claim 4, wherein said antibody is conjugated to a radioisotope label.
8. An antibody-conjugat according to claim 7, wherein said radioisotope label is techn tium-99m.
9. A therapeutic composition for treatment of angiogenesis-dependent disease, comprising an antibody according to

claim 1 or 2 or an antibody-conjugate according to any of claims 4 to 8, together with a pharmaceutically acceptabl carrier or diluent.

10. An antibody according to claim 1 or 2, or an antibody-conjugat according to any of claims 4 to 8 for us in a method of treating angiogenesis-dependent disease in a patient.

11. Use of an antibody according to claim 1 or 2 or an antibody-conjugate according to any of claims 4 to 8, in the manufacture of a pharmaceutical composition for treatment of angiogenesis-dependent disease.

12. An antibody or antibody-conjugate according to claim 10 for use in a method of inhibiting angiogenesis associated with the growth of solid tumours or with proliferative retinopathies.

13. Use according to claim 11 wherein the angiogenesis is angiogenesis associated with the growth of solid tumours or with proliferative retinopathies.

14. A method for producing an antibody according to claim 1 or 2 comprising raising antibodies against proliferating human umbilical vein endothelial cells (HUVEC), screening the antibodies for proliferating HUVEC reactivity, eliminating antibodies which react with other human cell lines, and identifying an antibody which fails to react with freshly isolated, non-proliferating human endothelial cells.

15. A method according to claim 14, which comprises eliminating antibodies which react with other proliferating human cell lines.

16. A method according to claim 15, wherein the proliferating human cell lines include a melanoma cell line.

Patentansprüche

1. Antikörper, der spezifisch an sich vermehrende humane Endothelzellen bindet, und an sich vermehrende humane Nabelvenenendothelzellen oder sich vermehrende humane Nabelarterienendothelzellen, die im Medium 199, ergänzt mit 20 % Rinderfetenserum, L-Glutamin, Antibiotika, 130 µg/ml Heparin und 1,2 µg/ml Endothelzellenvermehrungssupplement, kultiviert werden, bindet und nicht an sich nicht vermehrende humane Nabelvenenendothelzellen oder sich nicht vermehrende humane Nabelarterienendothelzellen bindet.

2. Antikörper nach Anspruch 1, der ein monoklonaler Antikörper ist.

3. Hybridomzelllinie, die einen monoklonalen Antikörper nach Anspruch 2 produziert.

4. Antikörperkonjugat, das einen Antikörper nach Anspruch 1 oder 2 mit einem daran gekoppelten toxischen Stoff oder mit einer daran gekoppelten Markierung umfasst.

5. Antikörperkonjugat nach Anspruch 4, wobei der Antikörper an einen cytotoxischen Stoff gekoppelt ist.

6. Antikörperkonjugat nach Anspruch 5, wobei der cytotoxische Stoff eine Ricin-A-Kette, Diphtherietoxin, Pseudomonas-Exotoxin-A oder Idarubicin ist.

7. Antikörperkonjugat nach Anspruch 4, wobei der Antikörper an eine aus einem Radionuklid bestehende Markierung gekoppelt ist.

8. Antikörperkonjugat nach Anspruch 7, wobei die radioaktive Markierung ^{99m}Technetium ist.

9. Therapeutische Zusammensetzung zur Behandlung einer Angiogenese-abhängigen Erkrankung, welche einen Antikörper nach Anspruch 1 oder 2 bzw. ein Antikörperkonjugat nach einem der Ansprüche 4 bis 8 zusammen mit einem pharmazeutisch verträglichen Träger oder Verdünnungsmittel umfasst.

10. Antikörper nach Anspruch 1 oder 2 bzw. Antikörperkonjugat nach einem der Ansprüche 4 bis 8 für die Verwendung in einem Verfahren zur Behandlung iner Angiogenese-abhängigen Erkrankung eines Patienten.

11. Verwendung eines Antikörpers nach Anspruch 1 oder 2 bzw. eines Antikörperkonjugats nach einem der Ansprüche 4 bis 8 für die Herstellung einer pharmazeutischen Zusammensetzung zur Behandlung einer Angiogenese-abhängigen Erkrankung.

12. Antikörper oder Antikörperkonjugat nach Anspruch 10 für die Verwendung in einem Verfahren zur Inhibierung der Angiogenese, die mit dem Wachstum solider Tumoren oder mit sich ausbreitenden Retinopathien verbunden ist.

13. Verwendung nach Anspruch 11, wobei die Angiogenese eine solche ist, die mit dem Wachstum solider Tumoren oder mit sich ausbreitenden Retinopathien verbunden ist.

14. Verfahren zur Herstellung eines Antikörpers nach Anspruch 1 oder 2, welches das Züchten von Antikörpern gegen sich vermehrende humane Nabelvenenendothelzellen (HUVEC), Screening der Antikörper auf Reaktivität gegen sich vermehrende HUVEC, Entfernen von Antikörpern, die mit anderen humanen Zelllinien reagieren, und Identifizieren eines Antikörpers, der nicht mit frisch isolierten, sich nicht vermehrenden humanen Endothelzellen reagiert, umfasst.

15. Verfahren nach Anspruch 14, welches das Entfernen von Antikörpern umfasst, die mit anderen sich vermehrenden humanen Zelllinien reagieren.

16. Verfahren nach Anspruch 15, wobei die sich vermehrenden humanen Zelllinien eine Melanomzelllinie umfassen.

Revendications

1. Anticorps qui se lie spécifiquement à des cellules endothéliales humaines en prolifération, ledit anticorps se liant à des cellules endothéliales de veine ombilicale humaine en prolifération ou à des cellules endothéliales d'artère ombilicale humaine en prolifération, cultivées dans du milieu 199 supplémenté avec 20 % de sérum de veau foetal, de la L-glutamine, des antibiotiques, 130 µg/ml d'héparine et 1,2 µg/ml de supplément de croissance de cellules endothéliales, et
ne se liant pas à des cellules endothéliales de veine ombilicale humaine qui ne sont pas en prolifération ou à des cellules endothéliales d'artère ombilicale humaine qui ne sont pas en prolifération.

2. Anticorps selon la revendication 1 qui est un anticorps monoclonal.

3. Lignée cellulaire d'hybridome produisant un anticorps monoclonal selon la revendication 2.

4. Conjugué d'anticorps comprenant un anticorps tel que revendiqué dans la revendication 1 ou 2 ayant un matériau toxique ou un marqueur conjugué à lui.

5. Conjugué d'anticorps selon la revendication 4, dans lequel ledit anticorps est conjugué à un matériau cytotoxique.

6. Conjugué d'anticorps selon la revendication 5, dans lequel ledit matériau cytotoxique est une chaîne A de ricine, une toxine diphtérique, une exotoxine A de Pseudomonas ou une idarubicine.

7. Conjugué d'anticorps selon la revendication 4, dans lequel ledit anticorps est conjugué à un marqueur isotope radioactif.

8. Conjugué d'anticorps selon la revendication 7, dans lequel ledit marqueur isotope radioactif est le technetium-99m.

9. Composition thérapeutique pour le traitement d'une maladie dépendante de l'angiogénèse, comprenant un anticorps selon la revendication 1 ou 2 ou un conjugué d'anticorps selon l'une quelconque des revendications 4 à 8, avec un support ou un diluant pharmaceutiquement acceptable.

10. Anticorps selon la revendication 1 ou 2, ou conjugué d'anticorps selon l'une quelconque des revendications 4 à 8 pour un utilisation dans une méthode de traitement d'une maladie dépendante de l'angiogénèse chez un patient.

11. Utilisation d'un anticorps selon la revendication 1 ou 2 ou d'un conjugué d'anticorps selon l'une quelconque des revendications 4 à 8, dans la fabrication d'une composition pharmaceutique pour le traitement d'une maladie dé-

pendante de l'angiogénèse.

12. Anticorps ou conjugué d'anticorps selon la revendication 10, destiné à être utilisé dans une méthode d'inhibition de l'angiogénèse associée à la croissance de tumeurs solides ou à des rétinopathies prolifératives.

13. Utilisation selon la revendication 11, dans laquelle l'angiogénèse est une angiogénèse associée à la croissance de tumeurs solides ou à des rétinopathies prolifératives.

14. Méthode de production d'un anticorps selon la revendication 1 ou 2, comprenant le fait de produire des anticorps contre des cellules endothéliales de veine ombilicale humaine en prolifération (HUVEC, human umbilical vein endothelial cells), de cribler les anticorps pour la réactivité avec les HUVEC en prolifération, d'éliminer les anticorps qui réagissent avec d'autres lignées cellulaires humaines, et d'identifier un anticorps qui ne réagit pas avec des cellules endothéliales humaines fraîchement isolées qui ne sont pas en prolifération.

15. Méthode selon la revendication 14, qui comprend l'élimination des anticorps qui réagissent avec d'autres lignées cellulaires humaines en prolifération.

16. Méthode selon la revendication 15, dans laquelle les lignées cellulaires humaines en prolifération comprennent une lignée cellulaire de mélanome.

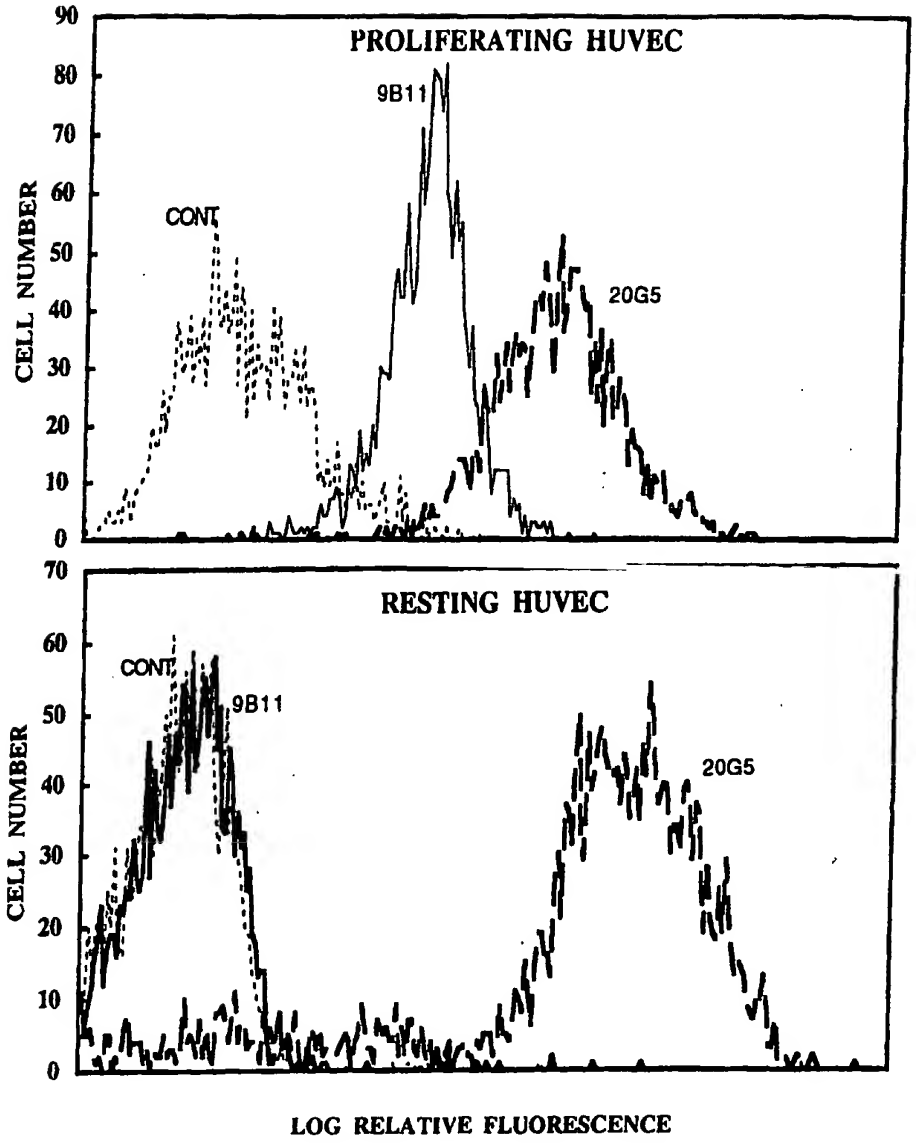
PROLIFERATING HUVEC
9B11
CONT
20G5
CELL NUMBER
90
80
70
60
50
40
30
20
10
0
RESTING HUVEC
CONT
9B11
20G5
CELL NUMBER
70
60
50
40
30
20
10
0
LOG RELATIVE FLUORESCENCE

Figure 1